Characterization of phenotypes based on severity of emphysema in chronic obstructive pulmonary disease

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Online Data Supplement

METHODS (online supplement)

Subjects

A total of 307 subjects with physician-diagnosed COPD were recruited at Hokkaido University Hospital and 9 affiliated hospitals from May 2003 through April 2005. All study protocols were approved by the ethics committees of all hospitals, and all subjects provided written informed consent. All were either current or former smokers with a smoking history of at least 10 pack-years. We carefully excluded the subjects when respiratory physicians diagnosed them as having bronchial asthma or bronchiectasis at the entry of this study. Subjects were also excluded when they had active tuberculosis, any history of lung cancer, any history of lung resection, and any history of cystic fibrosis, allergic alveolitis or pulmonary fibrosis. Those who would compromise 5-year follow-up or accurate assessment of pulmonary function or who were receiving long-term supplemental oxygen therapy for > 12 h/day were also excluded. To avoid interference with bronchodilator reversibility testing, those who had been using non-selective beta-blockers for treatment of hypertension were excluded. On the first visit, diagnoses were reconfirmed by pulmonologists and established based on the spirometric criteria of the GOLD guidelines.[E1] While 33 subjects were excluded from this study because the post-bronchodilator ratio of forced expiratory volume in 1 s (FEV_1): forced vital capacity (FVC) was ≥ 0.7, these subjects were encouraged to join the subsequent follow-up study.

Chronic bronchitis symptoms

Well-trained clinical research coordinators (CRCs) elicited disease history, smoking history and other information about all treatments. Chronic cough and sputum expectoration were considered to be present when they occurred on most days for ≥ 3 months/year and for ≥ 2 consecutive years.[E2,E3] To avoid a bias by patient reports about the presence of chronic sputum symptoms, the amount of sputum should be > 10 ml/day for the definition described above and this was confirmed by clinical research coordinators for all subjects.

Pulmonary Function Tests

Rolling seal spirometers, Chestac (Chest M.I., Inc., Tokyo) or Fudac (Fukuda Denshi CO., Ltd., Tokyo), were used for the measurement of spirometry and diffusing capacity of carbon monoxide (DLco) at all hospitals. The accuracy of spirometers had been examined everyday by trained technicians. Volume calibration using a calibration syringe had been performed by technicians in each hospital before examination, and maintained to acceptable standards for spirometry testing according to Japanese Respiratory Society (JRS) guidelines ,[E4] which are compatible with ATS recommendations.[E5] A quality-control protocol was developed based on the criteria used in the Lung Health Study to increase accuracy and decrease intra-individual variability.[E6] All technicians in each hospital were trained by a central coordinator, although they had extensive prior experience in pulmonary function testing. On the test day, we measured body mass index (BMI) and confirmed that subjects were in a stable condition and free from any medication that might influence Pulmonary Function Tests using following methods. The CRCs asked subject condition and confirmed freedom from respiratory medication by telephone on the previous day. All subjects had undergone a physical check by a respiratory physician just before spirometry to confirm subjects were stable and free from

respiratory infection. Finally, a technician reconfirmed to make sure omitting respiratory medicine. Inhalation of short-acting bronchodilators was prohibited in the 12 h preceding measurement, and long-acting β_2 adrenergic agonists or sustained-release theophyllines were prohibited in the previous 24 h. Only 2 subjects were using long-acting anticholinergic inhaled agents, as these agents had been not available in Japan until December 2004, and these subjects were asked to omit long-acting anticholinergic inhaled agents for 48 h before measurements. Acceptable maneuvers and acceptable measurements followed the guidelines for pulmonary function tests issued by JRS.[E4] We checked all the spirometric tracings from different centers in the central office. Predicted values of spirometric measurements were derived from the guidelines for pulmonary function tests issued by JRS.[E4]

We evaluated the reversibility of airflow limitation by measuring spirometry before and 30 min after inhalation of salbutamol (0.4 mg). Bronchodilator response (BDR) was expressed in three ways, as an absolute change in FEV_1, as a percentage change from baseline FEV_1, and as a percentage change from predicted FEV_1. Reversibility of airflow limitation was considered to be significant if an increase in FEV_1 was both > 200 ml and 12% above pre-bronchodilator FEV_1 according to GOLD guidelines.[E1]

DL_{CO} was measured by the single breath method. Results were corrected by alveolar volume (V_A) and hemoglobin concentration. DL_{CO} / V_A were compared to the predicted normal values.[E4]

HRCT

The following commercially available scanners were used in 9 affiliate hospitals: Somatom plus Volume Zoom (Siemens, Berlin); Aquilion Multi TSX-101A/2A, TSX-101A/4E, TSX-101A/6A, X Force TSX-011A/6A, X Vigor SS TSX-012A/4B (Toshiba Medical Systems, Japan); MX-8000/ID16 (Philips, Netherlands). Chest HRCT scans were performed under the supine position, holding their breaths at full inspiration. No patient received intravenous contrast medium. Other technical parameters were as follows; 1 mm collimation, 120-140 kV, 75-350 mA, 0.75-1 s for scanning time, and 1-2 mm thickness. HRCT images were selected at 3 levels, including the aortic arch, carina, and 1-2 cm above the highest hemi-diaphragm. Image interpretations were performed under -600 to -900 Hounsfield units (HU) window levels and 800 to 1500 HU window widths based on the best condition for detecting emphysema at each hospital.

Severity of emphysema was visually assessed by three independent pulmonologists according to the modified Goddard scoring system.[E7] We analyzed 6 images in 3 slices in the lungs and an average score of all images was considered as a representative value of severity of emphysema in each person. Each image was classified as normal (score 0), up to 5 percent affected (score 0.5), up to 25 percent affected (score1), up to 50 percent affected (score 2), up to 75 percent affected (score 3), more than 75 percent affected (score 4), giving a minimum score of 0 and maximum of 24, and the average score of 6 images was considered as a representative value of the severity of emphysema in the lungs. When the three independent pulmonologists split in their evaluation, we took only the score assessed by the majority.

Three dimensional CT analyses were performed only in Hokkaido University hospital (n=137) to confirm the accuracy and the reliability of visual assessment. All

CT row data sets were reconstructed to isometric voxel data using both soft-tissue and bone algorithms. The length of the 1 voxel side was 0.625 mm. Reconstructed data were transferred to the workstation, and then reconstructed into three-dimensional chest images (AZE Ltd, Tokyo, Japan). On this workstation, using original software with volume rendering technique, three dimensional lung objects for analyses were obtained at 3steps as follows; First, only trachea, bronchus and entire lung were extracted by using opacities under -740 HU. Second, trachea and central bronchus until second order bronchus were extracted from the object after the first step by using opacities from -740 to -975 HU. Third, only the entire lung object was obtained by subtraction of the second object from the first object. In these steps, the software automatically removed central pulmonary arteries and veins until the second order branch, which had similar opacities of lung, using original algorithm recognizing morphology. As in some previous reports, low attenuation volume (LAV) of the entire lung was measured as a volumes of the lung with attenuation coefficients lower than -960 HU.[E8,E9] LAV represents relative values in the total lung volume.[E10,E11]

The St. George's Respiratory Questionnaire (SGRQ)

SGRQ was used for assessment of health-related QOL. The SGRQ is a supervised, self-administered measure designed specifically for use in respiratory disease, and contains three domains: symptoms (relating to cough, sputum, wheeze and shortness of breath); activity (relating to physical activity limited by breathlessness); and impact (relating to control, panic, medication and expectations).[E12] A total score was calculated from all three domains.

Blood samplings

Blood was sampled from all subjects for the measurement of α_1-antitrypsin, leukocyte count, eosinophil count and immunoglobulin (Ig) E level.

Quality control

Considerable effort was made to ensure high-quality data and strict adherence to the protocol of the Hokkaido COPD cohort study. Physicians, CRCs, technicians and nurses from all hospitals underwent standardized training. CRCs confirmed before and after the visit whether procedures and performances were satisfactory and whether the quality of data was appropriate. Data entry used double-blind entry procedures with pre-programmed logical checks to ensure accuracy.

Statistical analysis

Data are shown as means ± standard error of the mean (SEM) unless otherwise specified. Skewed data were either transformed to logarithmic data or expressed as medians with interquartile ranges. Univariate analysis employed χ^2 tests for categorical variables and one-way analysis of variance for quantitative variables with Scheffe's test as a post-hoc test for multiple comparisons. Relationships between two variables of quantitative data were examined using Spearmen's tests. For BMI and SGRQ scores, the Jonckheere-Terpstra test was used to examine trends for three groups of subjects. All statistical tests were two-sided and values of $p<0.05$ were considered statistically significant. Data were analyzed using SPSS for Windows version 12 software (SPSS Japan, Tokyo).

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